The activity of a 100 mg of particles (dry weight) is 7.0 μM/min.

EXAMPLE 7

Preparation of chymotrypsin particles using Mx-170 as a crosslinking agent

1 cc of Sepharose (4 B, particle size 40-190μ) particles (55 mg dry weight) were impregnated with a dymotrypsin solution (4 mg/cc) containing β-phenyl propionate as inhibitor (7 mg/cc) in phosphate buffer (0.05 M, pH 7.0). The particles were then suspended with stirring in a solution of Mx-170 (10 mg/ml) in phosphate buffer (0.05 M, pH 7.0) for two hours. The particles were rinsed with HCl 10^{-3} M (to break up the enzyme-inhibitor complex) and buffer until no UV and no enzymatic activity were found in the wash water. The particles were packed into a column (diameter 1.7 cm, height 0.3 cm) and tested for their activity with Benzoyl-tyrosine-ethyl-ester (5.10^{-4}M). The product formed was measured in the effluent. The results are given in the following table:

Q ml/min	P μM/ml	V μM/min
0.43	0.186	0.087
0.7	0.127	0.09
0.8	0.108	0.08

The activity of 100 mg particles (dry weight) is 0.16 μM/min.

From the above examples it can be seen that the process of the present invention has been and/or can be used to prepare immobilized enzymes of the following categories:

1. Oxidoreductases such as catalase, glucose-oxidase;
2. Hydrolases such as proteases, i.e. Chymotrypsin, trypsin, Carboxypeptidase-B Amino-acid ester hydrolase amidases, i.e. asparaginase urease Carboxylic ester hydrolase, i.e. gluco amylase; and
3. Isomerases such as lysine racemase xylose isomerase; as well as other enzymes, such as those belonging to the classes of transferases, lyases and ligases.

Preferred is a process wherein an enzyme solution is pressed through the pores of a membrane and thereafter the enzyme loaded membrane is immersed into a solution of the cross-linking agents of the present invention, however as stated the process of the present invention can also be used to prepare immobilized proteins and enzymes for particle reactors and other uses which will readily suggest themselves to persons skilled in the art in light of the present description.

It will be evident to those skilled in the art that the invention is not limited to the details of the foregoing illustrative embodiments and examples and that the present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof, and it is therefore desired that the present embodiments be considered in all respects as illustrative and not restrictive, reference being made to the appended claims, rather than to the foregoing description, in which it is intended to claim all modifications coming within the scope and spirit of the invention.

What is claimed is:

1. A process for the cross-linking of proteins wherein the proteins to be cross-linked are reacted with a cross-linking agent selected from the group consisting of:

(a) water soluble poly(ethylene oxide) derivatives of the general formula I

$$(Z)_m—Y—(CH_2CH_2O)_nCH_2CH_2—Y—(Z)_m \quad (I)$$

wherein both n and m are at least 1; Y is a covalent bond or is an —R— or —RO— radical in which the oxygen is bound to the poly(ethylene oxide) and R is selected from the group consisting of methylene, ethylene, propylene, o—, m— and p-phenylene, o—, m— and p-phenylene carbamate optionally substituted by one or more alkyl, aryl, halo, nitro, oxo, carboxy, hydroxy, thio, sulfonate and phosphate groups; and Z is a reactive group selected from the groups consisting of haloisocyanato-, isothiocyanato-, tosylate, acyl halides, acyl azides, aryl diazonium salts, acyl imidoester salts, activated esters of acyl residues and 2,4-dichlorotriazines; and

(b) activated esters of di- and poly-carboxylic acids wherein the acids and the alcohol moieties thereof are water soluble, and wherein the alcohols forming the activated esters are selected from the group consisting of N-hydroxysuccinimide, 1-hydroxy benzotriazole, 8-hydroxyquinoline and 2- and 4-thiopyridine.

2. A process according to claim **1** wherein the proteins to be cross-linked are reacted with a cross-linking agent selected from the group consisting of poly(ethylene oxide) derivatives of the general formula I

$$(Z)_m—Y—(CH_2CH_2O)_nCH_2CH_2—Y—(Z)_m \quad (I)$$

wherein both n and m are at least 1; Y is a covalent bond or is an —R— or —RD— radical in which the oxygen is bound to the poly(ethylene oxide) and R is selected from the group consisting of methylene, ethylene, propylene, o-, m- and p-phenylene, o—, m— and p-phenylene carbamate optionally substituted by one or more alkyl, aryl, halo, nitro, oxo, carboxy, hydroxy, thio, sulfonate and phosphate groups; and Z is a reactive group selected from the groups consisting of halo-, isocyanato-, isothiocyanato-, tosylate, acyl halides, acyl azides, aryl diazonium salts, acyl imidoester salts, activated esters of acyl residues, and 2,4-dichlorotriazine.

3. A process according to claim **1** wherein the proteins to be cross-linked are reacted with a cross-linking agent selected from the group consisting of water soluble α,ω-dicarboxylic acid derivatives of poly(ethylene oxide) having the general formula II

$$X—\overset{O}{\overset{\|}{C}}—(Y')—(CH_2CH_2O)_nCH_2CH_2—(Y')—\overset{O}{\overset{\|}{C}}—X \quad (II)$$

wherein n is at least 1;

Y' is selected from the groups consisting of

(i) arylene carbamate, optionally substituted by one or more alkyl, aryl, halo and nitro groups

(ii)-$(CR_1R_2)_pO$ -; and

iii) $—(CR_1R_2)_p\overset{O}{\overset{\|}{C}}—O—$

in which p is at least 1, the oxygen is bound to the poly (ethylene oxide) and R_1 and R_2 are each selected from the group consisting of hydrogen and halogen atoms, nitro, and carboxyl groups and straight and branch chain alkyl and aryl groups optionally substituted by